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Case Report

Histoplasmosis: A case report

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ABSTRACT

Histoplasmosis is common in certain regions of America, Asia and Africa. Cutaneous manifestation of *Histoplasma capsulatum* shows a wide spectrum of lesions. Histopathology plays a very important role in the detection and confirmation of diagnosis. Special fungus stains such as GMS (Gomori's methenamine silver stain) and PAS (Periodic acid Schiff) stains can be used for its study.

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1. Introduction

Histoplasmosis stands as a significant global fungal ailment, stemming from the dimorphic fungus known as *Histoplasma capsulatum*.¹ Of the fungus' variations, *H. capsulatum* var. *capsulatum* (Hcc) and *H. capsulatum* var. *duboisii* (Hcd) prove harmful to humans.²

The infection is common in certain regions of America, Asia and Africa.³ Histoplasmosis attributable to Hcd finds its endemic roots in Africa.¹ The HIV and immunosuppressive therapies have resulted in more cases of Histoplasmosis.⁴⁻⁶ The severity of infection depends on the inoculum size, the virulence of the fungal strain, and the immune status of the host.⁷ Cutaneous manifestation shows a wide spectrum of lesions including erythematous plaques; maculopapules, crusted, verrucous or desquamative papules and nodules; abscesses; mucocutaneous ulcers; cellulitis to acneiform or varicelliform lesions; and molluscum-contagiosum like lesions.⁸ Symptoms commonly associated with Hcd infection include the presence of papules, nodules, ulcers, enlarged lymph nodes, as well as skin lesions resembling eczema or psoriasis.^{9,10} Additionally, subcutaneous abscesses may manifest, accompanied by

draining sinuses containing the fungus's yeast cells.¹

The infection is acquired by the inhalation of fungal spores usually present in the moist soils rich in nitrogen and acid containing excrement.¹¹⁻¹⁴ Therefore, occupational exposure to the fungus can occur during construction and caving activities. The exact pathogenesis of the Hcd infection remains unclear.¹ Acquisition may occur through the inhalation of microconidia or direct inoculation.¹ Despite the general belief of inhalation as the primary mode of acquisition, the lungs typically remain unaffected.¹ Disseminated forms of the infection often entail bone and multi-organ involvement, extending to the gastrointestinal tract.^{2,9}

Histoplasma capsulatum stands as the primary cause behind classical histoplasmosis, showcasing a fascinating dual nature as a fungal pathogen.^{15,16} It manifests in two distinct forms: a mould variant thriving in ambient temperatures and a yeast variant flourishing at body temperature.^{15,16} The fungus primarily inhabits soil enriched with bird or bat guano, predominantly existing in the mould form composed of hyaline septate hyphae, measuring 1 to 2.5 μm in diameter.^{15,16} These hyphae give rise to two distinct hyaline asexual reproduction structures: macroconidia and microconidia.^{15,16}

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Macroconidia, also known as tuberculate conidia, measure between 8 to 15 μm in diameter and bear a defining thick wall marked by unique projections on the surface.^{15,16} On the other hand, microconidia present themselves as minute, sleek structures measuring between 2 to 4 μm in diameter.^{15,16} These tiny microconidia possess the capability to lodge themselves in the alveoli upon inhalation, representing the infectious forms, while the larger macroconidia assist in the organism's identification.^{15,16}

The pathogenic form of *H. capsulatum* materializes in the yeast form, measuring between 2 to 4 μm in diameter.^{15,16} This form is typically found within the tissues of infected individuals or when the organism is cultivated at temperatures equal to or exceeding 37°C in vitro.^{15,16} Moreover, *Histoplasma duboisii*, produces larger yeast cells measuring between 8 to 15 μm in length.^{15,16} These distinctions highlight the diverse morphological variations of this fungal pathogen across different environments and geographical locations.^{15,16}

In summary, *Histoplasma capsulatum*, with its intricate morphological duality and variant manifestations, showcases a remarkable adaptability and prevalence in diverse environmental niches, accentuating the complexity of its pathogenic potential and geographical distribution.^{15,16}

Perinatal and congenital infections have been observed in children living with HIV, indicating a vulnerability to various infections.^{17,18} Histoplasmosis has not been uncommon among African children. Oladele et al. documented 37 pediatric cases out of 470 between 1952 and 2016 in a comprehensive review of histoplasmosis in Africa. Furthermore, Pakasa et al.'s case series study conducted in 2018, highlighted a substantial incidence of histoplasmosis (44.4%, 16 out of 36 cases) among young children aged 3 to 7 years.¹⁹ Amona et al.'s study done in 2021 revealed a significant proportion (31.5%) in the pediatric age group.²⁰

However, diagnosing histoplasmosis remains challenging. It often gets misidentified as other clinical conditions such as tuberculosis (TB), pneumonia, various cancers, nephrotic syndrome, and hyperreactive malarial splenomegaly syndrome.^{21–23}

This misdiagnosis and delay in identifying histoplasmosis contribute to prolonged hospital stays, financial burdens, wastage of time, inappropriate administration of antibiotics, and unfortunately, fatalities.^{26–28} Histoplasmosis, resembling TB or cancers in symptoms, often leads to a prioritization of these more common diseases in diagnosis, causing a delay in the proper identification and treatment of histoplasmosis cases.^{24–26}

The repercussions of such diagnostic challenges and delays are far-reaching, impacting not only the individual's health but also straining healthcare resources and potentially leading to severe outcomes including unnecessary treatment

courses and even loss of life.²⁷ Addressing these diagnostic hurdles is crucial to mitigate the negative consequences associated with misidentification and delay in treating histoplasmosis, especially in regions where it appears relatively frequently among pediatric populations.²⁷

Radiographic examinations, like chest x-rays or chest CT scans, serve as primary diagnostic tools for children exhibiting potential pulmonary conditions. These imaging procedures enable clinicians to initially investigate suspected pulmonary ailments.^{28–31} They provide comprehensive insights into the chest area, aiding in the identification and evaluation of potential abnormalities or diseases affecting the lungs or surrounding structures.^{28–31} Employing these radiological techniques at the outset of diagnosis allows for a detailed examination, facilitating accurate assessments and subsequent targeted treatment plans for paediatric patients presenting symptoms or indications of pulmonary illness.^{28–31} Histoplasmosis include lymphadenopathy (hilar, perihilar, mediastinal, subcarinal, and paratracheal), pulmonary nodules, consolidation, infiltrates, ground glass opacification, pleural effusion, and cavitary lesions. Bronchial or vascular compression, calcifications, and rarely, pericarditis may be seen.³¹

The gold standard in diagnosing childhood histoplasmosis remains the fungal culture, a microbiological method renowned for its accuracy.^{30,31} This diagnostic approach involves cultivating fungal specimens from diverse sources like sputum, bronchoalveolar lavage fluid, blood, or bone marrow aspirates.²⁷ Through meticulous culturing techniques, clinicians obtain definitive evidence aiding in the identification of histoplasmosis in paediatric patients. These various specimen sources allow for a comprehensive exploration, enhancing the likelihood of detecting the fungal presence and ensuring a more precise diagnosis. Employing fungal cultures from multiple sample types significantly amplifies the diagnostic capability for accurate identification of histoplasmosis in children.²⁷

Serologic tests are typically positive in up to 90–95% of all children with symptomatic histoplasmosis.³⁰ Agar gel immunodiffusion (ID) and complement fixation tests (CFTs) are the most commonly used.²⁷

Histopathology plays a significant role in the detection and confirmation of diagnosis.³ Presence of tiny 2- to 4- μm spores within the cytoplasm of macrophages and variably within giant cells is the diagnostic feature in all types of cutaneous histoplasmosis. The spores appear as round or oval bodies surrounded by a clear space.³² Special fungus stains are used for the study of mycotic diseases such as GMS (Gomori's methenamine silver stain) and PAS (Periodic acid Schiff) stains.³

Direct microscopy utilizing Giemsa stain has emerged as an intriguing method for diagnosing histoplasmosis, particularly in resource-constrained settings where the

accessibility and affordability of diagnostic tools pose significant challenges.²⁷ This technique was notably employed by Garcia-Guñón et al. in 2009, detailing a case of disseminated histoplasmosis in a 10-year-old.³³ However, the method's sensitivity remains a concern, as it may yield false-positive results, attributable to the microscopic challenge of differentiating *Histoplasma* from other yeast varieties like *Candida*, *Cryptococcus*, *P. brasiliensis*, *Pneumocystis jirovecii*, *Leishmania donovani*, and *Toxoplasma gondii*.³⁴

Despite its utility, the limitations in specificity underscore the need for complementary diagnostic approaches.²⁷ While Giemsa staining aids in preliminary identification, its susceptibility to misinterpretation necessitates corroborative tests for accurate histoplasmosis diagnosis.²⁷ In regions facing resource constraints, this method's cost-effectiveness renders it valuable, yet the potential for misdiagnosis accentuates the importance of employing a spectrum of diagnostic modalities to ensure precision in identifying this fungal infection, especially in pediatric cases where timely and accurate diagnosis significantly impacts patient outcomes.²⁷

2. Case Presentation

A 30 years male, from Aligarh presented to the Outpatient Department with complaints of multiple skin nodules over the body for 5 months. On physical examination, these were firm, mobile, 5-6 cm swelling.



Figure 1: Multiple skin nodules present over the body.

Skin biopsy was done from the lesions and sections were stained with routine H & E stain and PAS (special stain). H & E stained sections showed keratinized stratified squamous epithelium with underlying mid-dermis showing abundant mixed inflammatory cells along with histiocytes which showed intracytoplasmic spores (2-4 μm in size), round with clear space around them. PAS stain was positive.

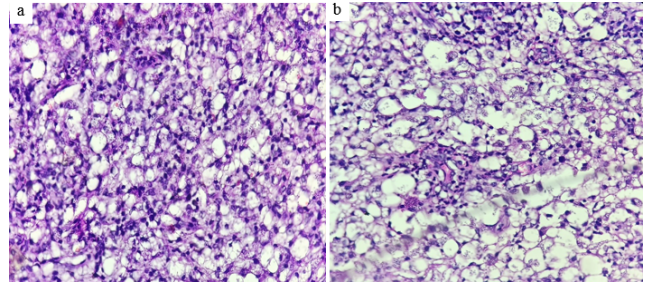


Figure 2: a and b: 40 X: Intracytoplasmic *Histoplasma* spores.

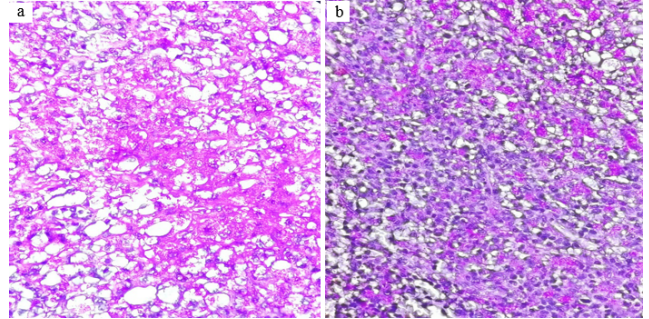


Figure 3: a and b: PAS positive spores of *Histoplasma*.

3. Discussion

Samuel first described *Histoplasma capsulatum* while investigating a case of miliary tuberculosis in 1906. The name *Histoplasma capsulatum* was coined due to its appearance as an encapsulated organism in histiocyte.³⁵ In India, the first case of histoplasmosis was diagnosed in 1954 by Panja and Sen from Kolkata.³⁶ Histoplasmosis is endemic in north east, West Bengal and Uttar Pradesh region of India due to their temperate climate and organic nitrogen rich soil.³⁷

A US study of 16 immunocompromised patients suggested that histology was the most useful diagnostic procedure for histoplasmosis.³⁸ On histopathology, several fungi can be confused with *H. capsulatum* var. *capsulatum*, i.e., *Cryptococcus*, *Talaromyces marneffeii*, *Blastomycosis dermatitidis*, and *Candida glabrata*.¹⁵ The diagnosis of cryptococcosis may be suggested by variations in spore size, a magenta-coloured rim on PAS, and mucicarmine owing to a mucopolysacchride capsule. *T. marneffeii* shows the formation of a transverse septum rather than budding. *B. dermatitidis* has admixed larger forms, a broad-based bud, and a thick cell wall (size 7–20 μm). *C. glabrata* may show more size variability than *Histoplasma*, and pseudohyphae, and the inflammation is mainly neutrophilic. In India, an important differential diagnosis is the presence of the LD bodies of *Leishmania donovani*, a protozoan. However, *H. capsulatum* can be differentiated from LD bodies by seeing budding and positivity on PAS and GMS stain that is not seen with LD bodies.³ Silver impregnation stains and

electron microscopic studies show that *H. capsulatum* does not have a capsule and the inner portion of the clear space represents the cell wall of the fungus and the clear space itself is filled with granular material that separates the cell wall of the fungus from the cytoplasm of the macrophage.³²

Confirming a diagnosis involves showcasing the presence of the fungal evidence within a laboratory setting. The primary method relied upon to detect *Histoplasma* infection typically involves histopathology.³⁹ However, the appearance of yeast cells bears resemblance to other endemic fungal infections like blastomycosis. Recent advancements in diagnosing African histoplasmosis have revealed considerable limitations in this traditional identification method.⁴⁰ In a retrospective study done by Valero et al in 2018 encompassing thirteen patients from Spain, the fungus was only detected through histopathological examination in 36% (4/11) of the cases tested, all of which were positive via qPCR.⁴⁰ Researchers have suggested that conventional methods based on measuring yeast size might not adequately differentiate between Hcd and Hcc.⁴⁰ Molecular analysis significantly contributes to confidently distinguishing between these two species.⁴⁰

Culturing remains the benchmark, yet it sporadically yields positive results.⁴¹ This raises concerns regarding the classification of certain cases as either possible or probable. Despite advancements in antigen testing for classical histoplasmosis, a similar pattern has not yet been established for Hcd infection. The efficacy of previously assessed and recommended assays for detecting *Histoplasma* antigens, as well as newly introduced ones, still awaits extensive implementation in diagnosing Hcd infection. Additionally, the current antigen detection assays lack species specificity, which poses a risk in regions like Africa where both Hcc and Hcd are prevalent, potentially leading to misdiagnosis between the two. Consequently, some cases identified as Hcd infection might have actually been classical histoplasmosis or other endemic fungal infections. This uncertainty arises from the reliance on histopathology or antigen detection methods, both of which possess questionable specificity.^{2,20}

While the gold standard for diagnosing histoplasmosis remains the culture of pathogens, histological examination holds greater significance in ensuring timely detection due to its shorter turnaround time of typically 3-5 days.⁴² This emphasizes the importance of considering direct microscopy and tissue biopsy as rapid and dependable methods in diagnosing histoplasmosis.^{43,44} It's crucial to note that initial examinations via direct microscopy in bone marrow smears may not always reveal *Histoplasma capsulatum*, potentially resulting in false negatives. To improve detection rates, repeating bone marrow punctures in multiple sites can enhance the chances of identification.⁴⁵

This was evident in a specific case where a patient's histoplasmosis diagnosis was only confirmed upon the third

examination via direct microscopy of bone marrow smears. This underscores the variability and potential limitations in initial diagnostics, necessitating a comprehensive approach and potentially repeated tests to achieve an accurate diagnosis. Therefore, while cultures remain the gold standard, the expedited nature of histological examinations, coupled with the need for vigilance in conducting and interpreting multiple tests, is paramount in ensuring a timely and accurate diagnosis of histoplasmosis.⁴⁵

4. Conclusion

This case report highlights that *Histoplasma* can present as multiple skin nodules and histopathology can be used to diagnose the fungus in such rare cases. Although, culture is the gold standard for diagnosis, histopathology is the commonest method deployed.

5. Conflict of Interest

None.

6. Source of Funding

None.


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